1/prts

The present invention relates to the use of propionic bacteria with a view to optimising the production of propionic acid and/or of propionates and, as the case may be, of acetic acid and/or of acetates at the colon.

For some years specialists in nutrition have been recommending to their patients a diet rich in fibres to which they attribute physiological and metabolic effects which may be beneficial to health.

It is known that dietary fibres resist the enzymatic digestion in the small intestine and are only degraded and assimilated at the colon, that is to say the terminal part of the intestine. Therefore the beneficial effect mentioned above can only be exerted if this degradation and this assimilation are as complete as possible at this preterminal location: the colon.

It has been possible to establish that these biological reactions result from the anaerobic fermentation of the dietary fibres under the action of micro-organisms in the colon. This fermentation culminates in the production of short-chain fatty acids, hydrogen, carbon dioxide and biomass.

These short-chain fatty acids are essentially acetic acid, propionic acid and butyric acid; in the healthy organism they can only be produced at the colon, since that is the only location in the human body where strict anaerobic conditions prevail which permit the fermentation at the base of their synthesis, with the exception of acetic acid of which a very small quantity may be produced in the hepatic region.

Different studies have proved the importance of these short-chain fatty acids which are beneficial to health.

According to the literature it would appear that the physiological roles of these three short-chain fatty acids would be different from one another: in effect, the acetic and propionic acids would be led directly towards the liver where the totality of the propionic acid would be

metabolised whilst a part of the acetic acid would then be led to different tissues, whilst the butyric acid would be used more specifically within the wall of the colon.

Therefore the synthesis of the short-chain fatty acids implies the presence in the colon, on the one hand, of a fibre-based substrate which is easy to provide through food and, on the other part, of a balanced and adapted bacterial flora which is present in an optimal and constant manner.

This bacterial flora can originate from either the persistent endogenous flora of each individual or from the diet.

It is in fact well known that the contents of the human digestive tract, which is specific to each individual and corresponds approximately to 1 to 1.5 kg of food material in the course of digestive transformation, contains a significant population of micro-organisms consisting of a mixture of numerous species which may be evaluated at 10^{11} to 10^{12} cells per gram in the colon; this population constitutes a bacterial mass of a certain weight of which the good or bad balance can only be modified radically and above all durably with difficulty through the simple fact of current diet.

However, the food which one ingests daily is never sterile and is therefore more or less charged with bacteria (milk, fermented milk products, cheese, cider, wine, beer, meat, etc.). Nevertheless, the modifications to the colic flora as a result of the absorption of these bacteria can only be temporary.

It may also be noted that it has already been proposed to attempt to modify the microbial population of the intestinal tract by the administration and in particular the voluntary ingestion of bacterial cells reputed to be beneficial to health (known as probiotics), particularly lactic bacteria or bifid bacteria.

The introduction into the organism of a significant population of these bacteria either by means of a particular diet or by the direct ingestion of these microbial cells has been proposed

more particularly with the aim of limiting the development of pathogenic and putrefying species; in fact it is known that the endogenous flora present in the colon is distributed into different bacterial groups of which certain are not harmful, and are indeed beneficial, whilst others, particularly clostridium and putrefying ones, lead to the production of toxic substances and have a negative influence on health.

The idea on which the invention is based consisted of introducing regularly into the organism, by the oral route, a significant quantity of a probiotic microbial flora capable of favouring the regular synthesis of short-chain fatty acids at the colon.

Amongst the microbial species which can be employed to this effect, lactic bacteria are not very well adapted since by their nature they produce first and foremost lactic acid and very secondarily acetic acid but not propionic acid nor butyric acid.

By contrast, bacteria of another type, the propionic bacteria, are capable of producing abundant quantities of propionic acid and acetic acid, the two short-chain fatty acids which are required to irrigate the tissue networks, for example in a percentage of 2/3 propionic acid to 1/3 acetic acid. These bacteria are present in cooked pressed cheeses; furthermore they have the advantage of being better equipped than the lactic bacteria to have an activity in the colon where the anaerobiosis is total and also of being more resistant to technological stresses than the lactic bacteria and the bifid bacteria.

It should be noted that it has already been proposed in the literature to cause the absorption of the propionic bacteria, in particular in order to stimulate the development of bifid bacteria in the intestine (document WO 97/19689) or to release nitrogen monoxide in the human or animal digestive tract (document WO 98/27991). However, until now no-one has ever had the idea of using these bacteria for the production of short-chain fatty acids at the colon.

Therefore the present invention relates to the use of propionic bacteria selected as a function of their nature which is not very autolytic and their ability to resist bile salts in order to obtain a current food composition or a dietetic or medicinal composition which is absorbable by a

human or an animal, prepared so that the bacteria are protected at least partially against gastric acidity, containing at least 10^6 cells/gram of these bacteria, capable of stimulating and increasing significantly the synthesis of propionic acid and/or of propionate and, as the case may be, of acetic acid and/or of acetate at the colon by anaerobic bacterial fermentation.

In order that these bacterial should have the expected beneficial effect it is essential to choose strains which are not very autolytic which are capable of reaching the colon without damage, possibly developing there and producing sufficient quantities of propionic acid.

It is well known that the two principal stresses to which the ingested bacteria are subjected during their passage in the upper part of the digestive tract are associated on the one hand with the acidity of the stomach environment (pH 4 to 1) and on the other hand with the presence of bile salts in the small intestine (of the order of 15 mmol/l at most at the duodenum).

It has proved possible to establish that bacterial which have been exposed to the stomach acidity are weakened and consequently incapable of resisting the bile salts, even if they remain viable at the exit from the stomach.

Consequently, in accordance with the invention it is essential to make the propionic bacteria undergo a treatment of a type which enables them not to undergo the gastric stress which as a general rule corresponds to an encapsulation which may be voluntary or involuntary for example in the case of a food of the cheese type.

This situation has been brought to light due to a test used to evaluate the influence of the acid pH and of the bile salts successively or individually on the viability of two strains of propionic milk bacteria belonging to the TL collection of the LRTL (Laboratoire de Recherches de Technologie Laitière – INRA of Rennes), namely the strains TL 162 and TL 24 belonging to the species *P. freudenreichii* subsp *shermanii*.

The results of this test are described below.

The bacteria were cultivated at 30°C on a YEL medium for 2 days (start of stationary phase). The optical density at 650 nm was respectively 2.28 and 2.64 for TL 162 and TL 24.

- *Acid stress*

The cultures were diluted to 1/10th in S medium (tryptone-lactate) at pH 2.5 (final pH 3.0). After incubation at 37°C for 45 minutes the cultures were centrifuged and the bacteria taken up in the same volume of YEL. The counts were made before and at the end of the incubation, and the resumption of the growth was followed by measurements of DO for 5 days at 37°C.

- *Bile stress*

The initial cultures were centrifuged and the bacteria taken up in a volume 10 times greater of YEL containing 0.3% of bovine bile (~50% of bile salts). After an incubation at 37°C for 90 minutes, the cultures were centrifuged and the bacteria taken up in the same volume of YEL. Counts were made before and at the end of the incubation, and the resumption of the growth was followed by measurements of DO for 5 days at 37°C.

- *Successive acid and bile stresses*

The bacteria were subjected to an acid stress as described previously, but after centrifugation the cells were taken up in YEL containing 0.3% bile. After a second incubation at 37°C for 90 minutes, the cultures were centrifuged and the bacteria taken up in the same volume of YEL. Counts were made before and at the end of the incubation, and the resumption of the growth was followed by measurements of DO for 5 days at 37°C.

The results obtained are reported, on the one hand, in Table 1 below which mentions the influence of the acid and/or bile stress on the viability of the bacteria and, on the other hand, in Figure 1 which is a diagram representing the resumption of the growth after the different stresses.

Table 1

		Viability (cfu/ml)		
		Before stress	After acid stress	After bile stress
Acid stress alone	TL 162	3.0×10^8	9.7×10^6	/
	TL 24	4.0×10^8	1.0×10^7	/
Bile stress alone	TL 162	3.0×10^8	/	4.4×10^8
	TL 24	4.0×10^8	/	4.7×10^8
Successive stresses	TL 162	3.0×10^8	9.7×10^6	2600
		4.0×10^8	1.0×10^7	< 10

It was also possible to establish that:

- the acidity involves a substantial mortality of the bacteria (96.8% for TL 162 and 97.5% for TL 124), which explains a sufficiently long delay for the resumption of the growth (Figure 1).

- the bile does not involve any mortality of the bacteria, hence a very rapid resumption of the growth.

- when the bacteria are subjected to the bile, after a previous acid stress, this leads to an almost total mortality of the bacteria. This result, totally unexpected, therefore indicates that bacteria which have undergone an acid stress and which nevertheless remain viable become totally sensitive to the bile, whilst without previous acid stress these same bacteria are totally resistant to the bile salts.

Taking this situation into account, pre-adaptation tests were carried out with the aim of increasing the resistance of the bacteria. It is known in fact that an acid pre-stress (pH 4.5 – 5) effectively protects the cells against an acid stress (pH 2).

Therefore three adaptation tests were carried out on TL 162:

- *acid pre-stress*: preliminary incubation of the cells at 37°C for 30 minutes at pH 5

- *bile pre-stress*: incubation for 30 minutes in the presence of 0.08% of bile

- *acid and bile pre-stress*: incubation for 30 minutes at pH 5 and in the presence of 0.08% of bile.

The same protocol was applied as previously and the results obtained are reported in Table 2 below:

Table 2

	Viability (cfu/ml)	
	Before stress	After successive stresses
Without pre-adaptation	3×10^8	2600
Acid pre-adaptation	3×10^8	100
Bile pre-adaptation	3×10^8	1400
Acid and bile pre-adaptation	3×10^8	< 100

Thus it was possible to establish that an acid pre-adaptation weakens the cells even more, whilst a bile pre-adaptation has no effect.

Therefore these results have made it possible to demonstrate the necessity of treating the stresses successively and not separately as described in the majority of studies which have been carried out in this field.

However, it may be supposed that *in vivo* the conditions are less drastic for the bacteria (buffer effect of the food in the stomach, lesser bactericidal effect of the bile salts in micellar form with the phospholipids).

Taking account of the foregoing, in order to increase the quantity of viable bacteria an improvement in their resistance to the acid pH is not effective since the bacteria remain sensitive to the effect of the bile salts.

On the other hand, by protecting the bacteria from the acid stress, in particular by ingesting them prepackaged in gastroresistant capsules, it is possible to find in the faeces bacteria which are naturally resistant to the bile at a high level of viability.

Taking account of these results, a complementary test was carried out in order to compare the ability of the different strains of propionic bacteria to produce significant quantities of propionic acid after having been put in contact with bile salts.

In this test 33 strains of propionic milk bacteria belonging to the TL collection of the LRTL (INRA of Rennes) were compared as to their ability to survive in the presence of bile and then to produce propionic acid:

- 20 strains belonging to the species *P. freudenreichii* subsp *shermanii*
- 6 strains belonging to the species *P. freudenreichii* subsp *freudenreichii*
- 7 strains belonging to the species *P. acidipropionici*

The operating protocol was as follows:

Starting cultures in stationary phase (2 to 3 days of culture in YEL medium incubated at 30°C) were diluted to $1/10^{th}$ in YEL medium containing 0.6% of bovine bile (approximately 7-8 mmol/l of bile salts). This concentration of bile was chosen in order best to discriminate between the strains and constitutes the content of bile salts of the same order as those encountered in the duodenum.

The dilutions were incubated at 37°C for 90 minutes, then centrifuged. The bacteria were taken up in the YEL medium (initial volume) and put back to incubate at 37°C.

After 24 hours of incubation the DO at 650 nm was measured in order to evaluate the resumption of growth. The supernatant was collected then frozen in order to determine the fatty acids.

For certain strains the experiment was repeated so as to confirm the results.

The values of the optical densities at 650 nm before the bile stress and 24 hours after the end of the stress are reported in Table 3 below:

Table 3

Strains:	DO before the bile stress (initial DO/10)	DO at 24 hours of incubation after the bile stress	% of DO at 24 h/initial DO
P. shermanii TL125	0.32	2.02	63
TL 134	0.29 – 0.26	2.60 – 2.04	90 – 78
TL 144	0.39	3.62	93
TL 146	0.27	1.56	58
TL 147	0.28	1.23	44
TL 148	0.23	0.04	2
TL 160	0.36 – 0.32	4.67 – 4.03	130 – 126
TL 167	0.26	1.05	40
TL 168	0.32	0.57	18
TL 4	0.32	0.29	9
TL 14	0.23	0.73	32
TL 17	0.29	0.55	19
TL 22	0.28	1.39	50
TL 24	0.28	1.65	59
TL 162	0.20	2.08	104
TL 34	0.26 – 0.27	3.40 – 3.87	131 – 143
TL 50	0.26	2.05	79
TL 61	0.23	1.31	57
TL 63	0.27 – 0.26	3.26 – 3.55	121 – 137
TL 40	0.30	1.46	49
P. freudenreichii TL 142	0.34 – 0.31	2.95 – 2.80	87 – 90
TL 3	0.24	2.66	111
TL 19	0.30	2.66	89
TL 37	0.23	1.79	78
TL 33	0.26	2.38	92
TL 64	0.29	0.31	11
P. acidipropionici TL 2	0.38	2.30	61
TL 9	0.34	2.29	67
TL 15	0.34	3.09	91
TL 54	0.34	1.39	41
TL 47	0.20	0.22	11
TL 223	0.29 – 0.38	2.59 – 2.91	89 – 77
TL 249	0.44	3.40	77

It should be noted that for certain strains the final DO is higher than the initial DO, which may be explained by the growth resumed at 37°C and not at 30°C as initially.

As a function of the results obtained, three groups of strains may be distinguished schematically:

- the strains which are distinguished from one another by a rapid resumption of growth, indicating a low mortality of the cells due to the bile (amongst them TL 34, TL 160, TL 63, TL 33, TL 15, TL 3, TL 162),

- the strains which have very low resistance to the bile, showing a very poor or zero resumption of growth (TL 148, TL 4, TL 64, TL 47).

- the intermediate strains characterised by a moderate mortality due to the bile (TL 146, TL 147, TL 167, TL 168, TL 14, TL 17, TL 22, TL 24, TL 61, TL 40, TL 54).

Only the strains having a ratio [(DO at 24 h/initial DO) x 100] greater than 60% (threshold chosen arbitrarily) were selected for the measurement of the lactate, of the acetate and of the propionate by HPLC in the frozen supernatants. The initial lactate content of the YEL medium was 11.4 g/l.

The concentrations of lactate, acetate and propionate of the supernatants recovered after 24 hours of incubation are set out in Table 4 below.

Table 4

Strains:	Lactate consumed	Acetate produced	Propionate produced
	(in g/l)		
P. shermanii TL 125	5.6	0.6	2.4
TL 134	8.9 – 7.3	0.9 – 0.9	4.0 – 3.4
TL 144	8.8	1.1	4.6
TL 160	9.9 – 8.8	1.3 – 1.1	4.7 – 4.6
TL 162	5.3	0.6	2.3
TL 34	9.7 – 10.1	1.0 – 1.2	4.9 – 4.7
TL 50	6.6	0.6	3.7
TL 63	9.1 – 10.0	1.0 1.1	4.4 – 4.4
P. freudenreichii TL 142	8.1 – 8.2	0.9 – 0.9	4.5 – 4.0
TL 3	7.8	0.9	3.6
TL 19	6.6	0.7	3.7
TL 37	5.5	0.6	2.5
TL 33	7.2	0.8	3.4
P. acidipropionici TL 2	2.7	0.4	1.5
TL 9	5.3	0.6	2.4
TL 15	3.7	0.4	2.0
TL 223	2.8 – 3.8	0.4 – 0.4	1.8 – 1.7
TL 249	5.2	0.6	3.2

This table shows that in a predictable manner the quantity of propionate produced is correlated with the degree of utilisation of the lactate.

Generally speaking, the strains belonging to the species *P. acidipropionici* produce less propionic acid than the strains of the species *P. freudenreichii* in 24 hours.

In view of the results reported above, certain strains prove to be better candidates as regards production of propionate after the action of the bile. These are strains producing at least 2 g/l of propionate in the conditions described above:

TL 134, TL 50, TL 3, TL 19. TL 33, TL 249,

and preferably more than 4 g/l of propionate:

TL 160, TL 144, TL 34, TL 63, TL 142

An experiment was also carried out on healthy volunteers with the aim of verifying the beneficial influence of gastroresistant capsules in order to improve the survival in the intestine of a strain of propionic cheese bacteria ingested in lyophilised form (TL162).

The experiment was carried out on 7 individuals in total, with 3 periods of treatment of 4 weeks, separated by intervals of 3 weeks.

Treatment 1 consisted of ingesting for 2 weeks 5×10^9 cfu/j of bacteria packaged in non-gastroresistant capsules.

Treatment 2 consisted of ingesting for 2 weeks 5×10^{10} cfu/j of bacteria packaged in non-gastroresistant capsules.

Treatment 3 consisted of ingesting for 2 weeks 5×10^9 cfu/j of bacteria packaged in gastroresistant capsules.

For each treatment 4 samples of faeces were taken in order to test for the propionic bacteria with the aid of a selective medium (Palpropiobac®, Standa-Industrie, mixed with 4 mg/l of metronidazole). The sampling dates were:

- S1: just before the period of ingestion,
- S2: 1 week after the start of ingestion,
- S3: 2 weeks after the start of ingestion,
- S4: 1 week after the end of the period of ingestion,
- HP (for the period 3): 3 weeks after the end of the period of ingestion.

During the entire experiment the volunteers could not consume cheese containing propionic bacteria in a significant quantity (Emmental, Comté, Leerdammer, Gruyères Suisses, ...) with the exception of cheese spreads.

Table 5 indicates the results of viability of the propionic bacteria in the faeces.

With conventional capsules, the dose of 5 x 10^9 cfu/j (Period 1) appears insufficient in order to find viable propionic bacteria in a significant quantity in all the volunteers. On the other hand, with the dose of 5 x 10^{10} cfu/j (Period 2) all the volunteers had more than 5 log cfu/g of viable propionic bacteria in the faeces from the first week of treatment. However, the maximum levels of viability observed with the doses are not different (~7 log).

The use of gastroresistant capsules (Period 3) improves the viability of the propionic bacteria in the faeces, notably in the volunteers in whom little were found at the time of the first treatment (vol. 1, 2 and 6). In fact, in relation to Period 2 the levels of viability obtained are on average equivalent.

- on the basis of 5 x 10^9 cfu/j the use of gastroresistant capsules sis therefore justified for a certain number of individuals (vol. 1, 2, 6), for the others they do not or only slightly improve the viability (vol. 3, 4 and 5),

- they provide results approximately equivalent to the conventional capsules containing 5 x 10^{10} cfu.

The short-chain fatty acids were measured in the faeces by gas chromatography. The quantities of propionate in the faeces are indicated in Table 6. For the statistical analysis, 2 groups of values were compared 2 by 2: the values corresponding to the samples of faeces where the propionic bacteria were not detected (< 4 log), for all treatments and periods combined and the values corresponding to the samples of faeces where the propionic bacteria were numbered at more than 6 log cfu/g. In the first case the average quantity of propionate is 5.06 + 2.56 µmol/g (n = 25) and in the second case it is 7.19 ± 3.18 µmol/g (n = 30). These 2 values are significantly different at $p < 0.02$ (Student test). For the other short-chain fatty acids there are no significant differences. Therefore this experiment shows that the presence of substantial quantities (> 6 log cfu/g) of propionic bacteria in the colon following the ingestion of TL 162 significantly increases the quantity of propionate in the faeces. However, since the strain TL 162 does not prove to be the best candidate for optimising the production

of propionic acid in the colon (cf. criteria for selection *in vitro*), it is probable that the results could be improved with a strain selected according to the aforementioned criteria.

Table 5 : Count of propionic bacteria in the faeces at the time of the 3 periods of treatment
(results in log cfu/g of fresh faeces)

		Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Vol. 7	average	s	n*
Period 1	S1	4,00	< 4	< 4	4,00	< 4	4,85	4,30	4,29	0,40	4/7
	S2	4,00	< 4	4,70	6,00	7,15	4,85	5,68	5,40	1,12	6/7
	S3	5,88	4,00	6,85	6,08	6,30	4,70	6,76	5,79	1,07	7/7
	S4	< 4	< 4	< 4	6,11	6,48	< 4	5,00	5,86	0,77	3/7
Period 2	S1	6,46	< 4	6,43	6,30	< 4			6,40	0,09	3/5
	S2	5,93	5,40	6,69	6,30	7,20			6,29	0,71	5/5
	S3	6,36	6,46	6,83	6,38	5,00			6,20	0,70	5/5
	S4	5,11	< 4	< 4	< 4	6,11			5,61	0,71	2/5
Period 3	S1	3,85	< 3	6,15	< 4	6,36	< 3		5,46	1,40	3/6
	S2	6,51	5,43	4,95	6,79	6,91	5,68		6,03	0,78	6/6
	S3	6,56	5,77	6,70	6,23	6,11	5,95		6,22	0,35	6/6
	S4	5,26	< 3	< 3	6,00	< 3	5,89		5,98	0,78	3/6
	HP	4,60	< 2	2,77	6,20	7,30	< 2		5,23	1,98	4/6

n* = number of individuals in which the propionic bacteria could be counted
shaded cells : all of the values higher than 6 log cfu/g.

Table 6 : Propionate concentrate in the fresh faeces (in μmol/g)

		Vol. 1	Vol. 2	Vol. 3	Vol. 4	Vol. 5	Vol. 6	Vol. 7
Period 1	S1	7,70	4,82	3,73	2,38		3,70	13,83
	S2	10,03	3,61	5,43	5,32	4,84	3,86	18,08
	S3	9,44	3,88	2,29	4,35	3,36	7,99	12,01
	S4	6,00	2,60	3,68	8,40	5,06	5,17	10,45
Period 2	S1	9,56	7,36	13,77	8,49	6,14		
	S2	7,12	1,86	5,26	5,82	3,72		
	S3	10,60	5,11	3,99	6,42	4,83		
	S4	19,16	3,12	3,58	2,16	7,08		
Period 3	S1	11,90	5,05	10,82	3,43	5,54	2,04	
	S2	9,28	8,34	7,82	6,45	7,28	7,56	
	S3	10,91	6,62	10,63	7,12	6,39	2,25	
	S4	11,93	2,89	8,91	3,41	6,15	5,20	
	HP		3,58			6,58	6,49	

shaded cells : all of the values corresponding to samples with the propionic bacteria higher than 6 log cfu/g

Taking the foregoing into account and in accordance with a preferred characteristic of the invention, the propionic bacteria used are chosen from amongst the strains producing propionic acid in a physiologically significant quantity and in particular from amongst the strains producing at least 2 g/l of propionic acid and/or of propionates and. preferably, more than 4 g/l of propionic acid and/or of propionates after having been cultivated at 30°C in YEL medium containing approximately 11.4 g/l of lactate for 2 to 3 days, then diluted to $1/10^{th}$ in a YEL medium containing 0.6% bovine bile, incubated at 37°C for 90 minutes, centrifuged, taken up in YEL medium and put back to incubate at 37°C for 24 hours.

Another selection criterion which could be taken into account in accordance with the invention corresponds to the properties of adhesion of the strains on the colonocytes: strains having good properties of adhesion in effect have the advantage of staying longer in the colon, which allows them more time to synthesise the propionic acid; moreover, the strains which become fixed can take the place of the pathogenic agents.

It should be noted that in order to obtain the effect which is sought it is not envisaged to cause the propionic acid itself to be absorbed since because of the human metabolic chain it would not be able to reach the colon and furthermore it has been shown that in high doses it is harmful to the stomach.

Amongst the beneficial effects attributed to the short-chain fatty acids synthesised at the colon and in particular to acetic acid and above all to propionic acid, one may note their role in the assimilation of the principal minerals and notably calcium iron, zinc or even magnesium; in effect it has been possible to establish that propionic acid and, to a lesser extent, acetic acid can favour the colic absorption of these minerals and the use of the absorbed fraction by the organism.

That is a particularly interesting effect in view of the fact that the assimilation of minerals is accompanied by functional effects such as by way of example the correction of anaemia for iron or bone mineralisation for calcium.

The experimental and clinical studies which have been carried out provide a body of corroborating arguments in support of a favourable effect of propionic acid and propionates and, to a lesser extent, acetic acid and acetates on the metabolism of these minerals; this effect is probably more substantial when the conditions of digestion are poor, which leads to a substantial quantity of unabsorbed minerals being brought through the small intestine to the colic region, and when the needs are high.

The existence of correlations between the short-chain fatty acids and the metabolism of minerals has been suggested in particular by studies using soluble fibres. Thus it has been possible to establish that polysaccharides or oligosaccharides not digested by the digestive enzymes and which are therefore fermented into short-chain fatty acids (notably propionates) by the colic flora increase the absorption of minerals such as calcium, iron or zinc and that this increase is all the more marked if the conditions are pathological (deficiencies, gastrectomy ...).

Studies of colic perfusion and on the other hand the absence of effect in colectomised subjects has made it possible to confirm the localisation of the site of action at the colon.

It has also been confirmed that these actions are accompanied by a lowering of the pH and a synthesis of short-chain fatty acids; this suggests the intervention of these acids by means of a fermentation, all the more so since it has been verified that the insoluble fibres, which are not fermentable, have no effect. Furthermore, it has been established that the caecum is hypertrophied and that the colic blood flow increases, which attests to a trophic effect.

The clinical studies carried out on this subject are few, but they enable it to be confirmed that the effects of the soluble fibres are obtained by colic fermentation and show directly the effect of the short-chain fatty acids on the absorption of minerals.

Amongst these studies mention may be made of the publication *Trinidad TP, Wolever TMS, Thompson LU, Effect of acetate and propionate on calcium absorption from the rectum ad distal colon of humans, Am J Clin Nutr 1996 63/ 574-578* which reports on tests in which the

distal colon of healthy subjects was perfused directly with acetic acid, propionic acid or their association in physiological concentration; thus it was established that the disappearance of calcium from the colic orifice is increased by the short short-chain fatty acids, but in a significantly more substantial way by propionic acid; this study also showed a dose effect based on a non-saturable system of absorption. The authors suggested that the greater lipophilia of propionic acid compared with acetic acid could favour its absorption and the liberation in the colonocyte of protons whose passage into the digestive orifice would favour the absorption of calcium.

Taking account of the foregoing, the invention also relates to the use of propionic bacteria selected as a function of their nature which is not very autolytic and their ability to resist bile salts in order to obtain a current food composition or a dietetic or medicinal composition which is absorbable by a human or an animal, prepared so that the bacteria are protected at least partially against gastric acidity, containing at least 10^6 cells/gram of these bacteria, capable of favouring the assimilation of the principal minerals, in particular calcium and/or iron and/or zinc and/or magnesium at the colon.

According to a variant of the invention, it is also proposed to apply this use to obtaining a composition having antifungal properties at the colon and, in particular, capable of reducing the development there of pathogenic mycoderms of the candida/thrush type.

In fact this use takes advantage of the excellent antifungal properties of propionic acid particularly for the treatment of candidoses due to antibiotics.

It should be noted that the composition used according to the invention can, as the case may be, contain other bacteria, in particular lactic bacteria and/or bifid bacteria capable of acting in synergy with the propionic bacteria in such a way as to increase the effects mentioned above by supplying them with lactate as fermentable substrate.

The composition used according to the invention may consist of a dry or hydrated preparation presented in the form of individual fractions of approximately 100 mg to 1 g, preferably from

200 to 500 mg, preferably containing at least 10^8 cells; in particular it may advantageously be presented in the form of gastroresistant capsules.

According to another characteristic of the invention, this composition may also consist of a formulated preparation, the propionic bacteria being added to or associated with a fermentable substrate, notably dietary fibres, or added or incorporated into liquid, paste or solid foods.

In such a preparation the propionic bacteria can play a dual role, namely technological in a first period through the fermentation of food and function in a second period since once ingested they are capable of reaching the colon and there playing the aforementioned probiotic role, particularly as regards the optimisation of the synthesis of propionic acid and the optimisation of the assimilation of minerals.